



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: LIPOSOMAL ENCAPSULATED NUCLEIC ACID-COMPLEXES

(57) Abstract

This invention relates to liposomes which are useful for the introduction of nucleic acids into cells. The liposomes of the present invention entrap a condensing agent-nucleic acid complex and are suitable for nucleic acid-transfer delivery vehicles in clinical use. In addition, methods of transfecting a cell with a nucleic acid using the liposomes of the present invention are also disclosed.

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/16166

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/127 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 34647 A (VANDERBILT UNIVERSITY, U.S.A.) 21 December 1995 (1995-12-21) claims 1,2,4,5,9,11,15-17,19,20,22,23,27,30	1-54
X	WO 98 20857 A (UNIVERSITY OF CALIFORNIA) 22 May 1998 (1998-05-22) claims	1-66
X, P	WO 98 34648 A (RHONE-POULENC RORER) 13 August 1998 (1998-08-13) claims	1-66
E	WO 99 58694 A (UNIVERSITY OF CALIFORNIA) 18 November 1999 (1999-11-18) claims	1-66

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the International search

Date of mailing of the International search report

10 March 2000

16/03/2000

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/16166

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 32-54 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos.:

because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/16166

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9534647 A	21-12-1995	AU	2773695 A	05-01-1996
WO 9820857 A	22-05-1998	AU EP	7177998 A 0956001 A	03-06-1998 17-11-1999
WO 9834648 A	13-08-1998	FR AU CZ NO ZA	2759298 A 6298798 A 9902821 A 993825 A 9801034 A	14-08-1998 26-08-1998 13-10-1999 09-08-1999 11-08-1998
WO 9958694 A	18-11-1999	NONE		

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16303-72-1PC	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/16166	International filing date (day/month/year) 16/07/1999	Priority date (day/month/year) 20/07/1998	
International Patent Classification (IPC) or national classification and IPC A61K9/127			
Applicant INEX PHARMACEUTICALS CORPORATION et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 18/02/2000	Date of completion of this report 13.10.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Smeets, D Telephone No. +49 89 2399 7492



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/16166

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-29 as originally filed

Claims, No.:

1-66 as originally filed

Drawings, sheets:

1/15-15/15 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

the entire international application.
 claims Nos. 32-54 concerning industrial applicability and claims 65, 66 concerning novelty, inventive step and industrial applicability.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/16166

because:

- the said international application, or the said claims Nos. 32-54 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

- the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 65, 66 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

- the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 9-11, 14, 19-25, 28, 29, 40-42, 47-51, 53, 54, 56, 58, 61, 63, 64
	No:	Claims 1-8, 12, 13, 15-18, 26, 27, 30-39, 43-46, 52, 55, 57, 59, 60, 62
Inventive step (IS)	Yes:	Claims 9, 10, 19-25, 28, 29, 40, 47-51, 53, 54, 61, 63, 64
	No:	Claims 1-8, 11-18, 26, 27, 30-39, 41-46, 52, 55-60, 62
Industrial applicability (IA)	Yes:	Claims 1-31, 55-64
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/16166

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 32-54 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

The subject-matter of dependent claims 65 and 66 is unclear since in independent claim 55, no step a) or step b) are mentioned. See also **Re Item VIII** concerning the support of said claims by the description. Therefore, no opinion can be formed with regard to novelty, inventive step and industrial applicability.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: WO 95 34647 A (VANDERBILT UNIVERSITY, U.S.A.) 21 December 1995
(1995-12-21)
- D2: WO 98 20857 A (UNIVERSITY OF CALIFORNIA) 22 May 1998 (1998-05-22)

1) Remarks concerning claims 32-54 with regard to industrial applicability

For the assessment of the present claims 32-54 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2) Novelty - Art.33(1) and (2) PCT

The subject-matter of claims 1-8, 12-13, 15-17 lacks novelty in view of D2 (page 34, lines 10-21 page 35, lines 1-21, page 42, lines 10-25, page 44, lines 1-10, 15-18, page 45, lines 32-35, page 49, lines 19-25, 30-32), since said document discloses all the technical features of said claims.

The term "encapsulated" is not considered to be novel since no essential technical feature is present that distinguishes the subject-matter of said claims from D2 (page 34, lines 10-21 page 35, lines 1-21, page 42, lines 10-25, page 44, lines 1-10, 15-18, page 45, lines 32-35, page 49, lines 19-25, 30-32).

The subject-matter of claim 18 is not new in view of D2 (page 17, lines 12-13, 25-30) since said document discloses that PEG may also be derivatised with sphingolipids in order to increase the circulation lifetime of the lipid:nucleic acid complexes.

The subject-matter of claims 26-27 is not new in the light of D2 (page 42, lines 10-25) since the lipid:nucleic acid weight ratio, disclosed in said document, falls within the scope of claims 26-27.

The subject-matter of claims 30-31 is not new in view of D2 (page 34, lines 10-21 page 35, lines 1-21, page 42, lines 10-25, page 44, lines 1-10, 15-18, page 45, lines 32-35, page 49, lines 19-25, 30-32) since in said claims no additional technical features are defined in comparison with claim 1. The amount of encapsulation is considered to be an effect and the technical features for achieving this result are already disclosed in D2.

The subject-matter of claims 32-39, 43-46, 52 lacks novelty in view of D2 (page 34, lines 10-21, page 35, lines 1-21, page 42, lines 10-25, page 44, lines 1-10, 15-18, page 45, lines 32-35, page 49, lines 19-25, 30-32) since said document contains all the technical features of said claims.

The subject-matter of claims 55, 57, 59, 60 and 62 is not new in view of D2 (page 34, lines 10-21, page 35, lines 1-21, page 42, lines 10-25, page 44, lines 1-10, 15-

18, page 45, lines 32-35), since said document discloses all the technical features of said claims.

The subject-matter of claims 9-11, 14, 19-25, 28-29, 40-42, 47-51, 53-54, 56, 58, 61, 63-64 is new in view of the documents of the search report.

3) inventive Step - Art. 33(1) and (3) PCT

D2 is being regarded as closest prior art.

The technical problem of this invention is providing an alternative condensing agent-nucleic acid complex that is effective for facilitating intracellular delivery of genetic material but that will reduce the associated cellular toxicity.

D2 provides a solution by a composition comprising a condensing agent-nucleic acid complex incorporated in a liposome. Spermidine and spermine are used as condensing agents.

D1 (page 26, lines 24-25) provides an alternative solution by a histone protein as the condensing agent.

Claim 9, 40 and 61 provide a solution by using polyethylenimine as the condensing agent.

There are no indications in the closest prior art, nor in the other prior art that would prompt the skilled person, faced with the technical problem and the prior art to adapt or modify the solutions of the prior art to arrive at something falling within the scope of said claims. Therefore, the solution to this problem proposed in said claims of the present application is considered as involving an inventive step in view of the documents of the search report (Article 33(3)).

Claims 10, 23-25 and claims 50-51 are dependent respectively on claim 9 and claim 40 and as such also meet the requirements of the PCT with respect to inventive step.

The subject-matter of claims 11, 14, 41, 42 and 58 is not considered to involve an inventive step since it cannot be seen which technical problem is solved by having a complex diameter between 30 nm and 60 nm and a liposome diameter between 70 nm and 80 nm.

The subject-matter of claims 19-22, 28-29 and 47-49, 53-54, 63-64 involves an inventive step since there are no indications in the closest prior art, nor in the other prior art that would prompt the skilled person, faced with the technical problem and the prior art to adapt or modify the solutions of the prior art to arrive at something falling within the scope of said claims.

The subject-matter of claim 56 is not considered to involve an inventive step since it cannot be seen which problem is solved by admixing a first condensing agent to form a precondensed nucleic acid and then adding said precondensed nucleic acid into a second condensing agent.

D2 (page 35, lines 15-21) discloses that the optimal amount of polyamines (= condensing agent) is determined by titrating polyamines to the DNA.

If claim 56 intends to solve this problem, the feature of said claim is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

Re Item VII

Certain defects in the international application

- 1) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.
- 2) The term "incorporated herein by reference" (e.g. page 7, 9, 11, 12, 15, 22, 29) is not allowed as the application should be self-understanding (Art. 5, Rule 9.1 iv PCT).
- 3) The vague and imprecise statement in the description "spirit of the invention" (page 29, line 31) implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

Re Item VIII

Certain observations on the international application

1) Claims 65 and 66 are unclear since they contain a reference to a process with different steps a), b) and c) that are not mentioned in the independent claim 55 (Art. 6 PCT).

The description of the present application (page 14, lines 4-13) discloses a process with a step c). However, claims 65 and 66 are not supported by the description as required by Article 6 PCT. Detergent dialysis and ethanol injection are disclosed in the description of the present application (page 17, line 20 - page 18, line 22), but these techniques refer to the "encapsulation" of the complexes. Step (c) (description, page 14, lines 9-10) describes the dialyzing of the condensed nucleic acid complex to form a concentrated condensing agent-nucleic acid complex.

2) The feature of claims 9 and 40, that polyethylenimine has a molecular weight of 10 kDa to 50 kDa, is not referred to in the description. Claims 9 and 40 are therefore not supported by the description as required by Article 6 PCT.

3) The term "about" used in claims 9-14, 19-31, 40-42, 47-54, 58, 62-64 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).